Ecological Applications, 28(5), 2018, pp. 1342–1353

Selective and context-dependent effects of chemical stress across trophic levels at the basis of marine food webs

Christoph Mensens,[1,2] Frederik De Laender,[3] Colin R. Janssen,[2] Frances Camille Rivera,[1] Koen Sabbe,[4] and Marleen De Troch[1,5]

[1]*Biology Department, Marine Biology, Ghent University, Krijgslaan 281 – S8, 9000 Ghent, Belgium*
[2]*Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Coupure Links 653, Building F, 9000 Ghent, Belgium*
[3]*Research Unit in Environmental and Evolutionary Biology, Biology Department, Université de Namur, Rue de Bruxelles 61, 5000 Namur, Belgium*
[4]*Biology Department, Protistology and Aquatic Ecology, Ghent University, Krijgslaan 281 – S8, 9000 Ghent, Belgium*

Abstract. Human activities increasingly impact the functioning of marine food webs, but anthropogenic stressors are seldom included in ecological study designs. Diet quality, as distinct from just diet quantity, has moreover rarely been highlighted in food web studies in a stress context. We measured the effects of metal and pesticide stress (copper and atrazine) on the contribution of a benthic intertidal diatom community to two processes that are key to the functioning of intertidal systems: biomass (diet quantity) and lipid (diet quality) production. We then examined if stressors affected diatom functioning by selectively targeting the species contributing most to functioning (selective stress effects) or by changing the species' functional contribution (context-dependent effects). Finally, we tested if stress-induced changes in diet quality altered the energy flow to the diatoms' main grazers (harpacticoid copepods). Diatom diet quantity was reduced by metal stress but not by low pesticide levels due to the presence of an atrazine-tolerant, mixotrophic species. Selective effects of the pesticide reduced diatom diet quality by 60% and 75% at low and high pesticide levels respectively, by shifting diatom community structure from dominance by lipid-rich species toward dominance by an atrazine-tolerant, but lipid-poor, species. Context-dependent effects did not affect individual diatom lipid content at low levels of both stressors, but caused diatoms to lose 40% of their lipids at high copper stress. Stress-induced changes in diet quality predicted the energy flow from the diatoms to their copepod consumers, which lost half of their lipids when feeding on diatoms grown under low and high pesticide and high metal stress. Selective pesticide effects were a more important threat for trophic energy transfer than context-dependent effects of both stressors, with shifts in diatom community structure affecting the energy flow to their copepod grazers at stress levels where no changes in diatom lipid content were detected.

Key words: *atrazine; chemical stress; copepods; copper; diatoms; energy flow; fatty acids; marine food webs.*

Introduction

The impact of human activities on biological communities and their contribution to ecosystem functioning has become a central topic in ecological research (Halpern et al. 2008, Cardinale et al. 2012, Gamfeldt et al. 2015). Although conservation research is framed within the context of anthropogenic change, exposure to anthropogenic stressors however is rarely included in the design of studies focusing on biodiversity effects on ecosystem functioning (McMahon et al. 2012, De Laender et al. 2016).

Stress can affect ecosystem functioning by causing biodiversity loss in terms of species richness, as well as through changes in community structure, without necessarily causing species to go extinct (Hillebrand et al. 2008, Wittebolle et al. 2009, Mensens et al. 2015, De Laender et al. 2016). Selective stress effects on community structure (hereafter "selective stress effects"; Wittebolle et al. 2009) can influence ecosystem functioning if stressed communities are dominated by tolerant species with a low functional contribution (Larsen et al. 2005, Mensens et al. 2015). If the functionally most important species are also the most stress-tolerant, loss of functioning under stress will be limited (Radchuk et al. 2016). In addition, functioning in stressed communities can be altered by "context-dependent effects," i.e., changes in the species' functional contribution (Fox 2006, Fox and Harpole 2008, Tylianakis et al. 2008b, Hiddink et al. 2009). Context-dependent effects can arise from direct effects of the environmental drivers on the species' functional contribution (Fox and Harpole 2008; e.g., physiological stress; Schimel et al. 2007), as well as from environmental drivers altering species interactions (Fox 2006, Fox and Harpole 2008).

The majority of experiments designed to address ecosystem functioning under anthropogenic change have focused on single trophic levels, usually primary producers (Raffaelli 2006, Cardinale et al. 2011). Stressors that alter functioning at the producer level can however have concomitant impacts on their consumers (Rohr and Crumrine 2005, McMahon et al. 2012). In the frame of the subject of quantity of available food, some recent studies proposed methods to evaluate the abundance of trophic resources also according to the impacts of anthropogenic stressors (Zupo et al. 2017). Far less attention has been devoted to diet quality, which considers a diet's biochemical composition, for example lipid content (Jodice et al. 2006, Guo et al. 2016), and which strongly

Manuscript received 18 April 2017; revised 1 November 2017; accepted 6 April 2018. Corresponding Editor: Stephen B. Baines.
[5]Corresponding author. E-mail: marleen.detroch@ugent.be

affects growth, reproduction and energy profiles of the consumers (Österblom et al. 2008, Taipale et al. 2013). The biochemical composition of primary producers is increasingly affected by human disturbance (Guo et al. 2016, Sanpera-Calbet et al. 2017), which has made the integration of food biochemistry into traditional studies of diet quantity a key challenge for estimating food web functioning under stress (Arts and Wainmann 1999, Guo et al. 2016).

Benthic diatoms are the main primary producers in many soft-sediment intertidal habitats, and diatom diet quality in terms of essential fatty acid (EFA) content plays a crucial role in trophic energy transfer (Arts et al. 2001, Taipale et al. 2013). EFAs cannot be synthesized by animals but are key determinants of the growth and energy content of aquatic consumers (Brett and Müller-Navarra 1997, von Elert 2002, Arendt et al. 2005, Litzow et al. 2006). Moreover, the oxidation of polyunsaturated C16- and C20-FAs (polyunsaturated Fas or PUFAs) to short-chain polyunsaturated aldehydes (PUAs; Miralto et al. 1999) and other non-volatile oxylipins (NVOs), such as hydroxy-fatty acids, epoxy-hydroxy-fatty acids, and oxo-acids (D'Ippolito et al. 2005) can cause deleterious effects of diatom diets for consumers. Here, we first measure the effects of metal and pesticide stress (copper and atrazine, see further) on the structure of a benthic diatom community, its biomass and its production of essential fatty acids, i.e., on the quantity and quality of the diatoms as a diet.

Next, we test if stressor-induced changes in EFA production in the diatom community were caused by selective or context-dependent stress effects. Context-dependent effects are measured by comparing EFA concentrations in experimental diatom communities with those in synthetic communities. The latter are computed for all control and stress treatments from the individual species' EFA production values in unstressed monocultures, and as such uniquely mimic the selective stress effects while excluding all context-dependent effects.

Harpacticoid copepods are among the main consumers of benthic diatoms and incorporate large amounts of EFAs from their algal diet, making them key players in the energy transfer from primary producers to higher trophic levels (Alheit and Scheibel 1982, Buffan-Dubau and Carman 2000, Andersen et al. 2005). Therefore, any potential change in grazer EFA content, due to context-dependent stress effects on diatom diet quality or due to selective effects leading to dominance of diatoms with high or low lipid content, could impact the energy flow in intertidal systems. Therefore, we test if changes in survival and EFA content of the harpacticoid copepod *Microarthridion littorale*, the dominant copepod species at the study site, are related to atrazine and copper effects on the quality of its diatom diet. Next to its dominance in the study area, this harpacticoid copepod is an efficient grazer on epipelic diatoms forming a biofilm on the intertidal sediment in the study site and is relatively easy to manipulate in lab experiments (De Troch et al. 2012b).

Atrazine is an herbicide that binds to the plastoquinone binding protein of photosystem II, disrupting photosynthetic electron flow (Legrand et al. 2006, Knauert 2008) and thus the growth and photosynthesis of aquatic primary producers, such as microalgae (Pennington et al. 2001, Larras et al. 2016). Atrazine is commonly not acutely toxic to aquatic consumers, but has adverse chronic effects on consumers due to food limitation, hormonal disruption, and reduced reproduction, although these reproductive and hormonal effects are not consistently observed (Hayes et al. 2011). Despite its Europe-wide ban in 2001, atrazine is still a common pollutant in European estuaries (Noppe et al. 2007) and remains one of the most-used pesticides worldwide (Benbrook 2016). In contrast to organic pesticides, heavy metals occur naturally in the environment, and several of them are essential for organism physiology (Hänsch and Mendel 2009). This is the case for copper, which is involved in several metabolic pathways in microalgae, as an essential micronutrient and component of proteins and enzymes (Hänsch and Mendel 2009). However, copper concentrations above the required levels are toxic to marine organisms at all trophic levels (Real et al. 2003, Manimaran et al. 2012). As copper enters coastal environments through river run-off, it affects both primary producers and consumers through the formation of reactive oxygen species (ROS), which can lead to cell death by damaging cell membranes and nucleic acids (Rhee et al. 2013). Copper also affects marine primary consumers by inhibiting membrane transport proteins (Bianchini et al. 2004) and by limiting the quantity of their algal diet (Pinho et al. 2007).

Methods

Experimental organisms and culture conditions

The harpacticoid copepod *Microarthridion littorale* (family Tachidiidae) was collected from intertidal mud at the polyhaline Paulina site in the Westerschelde estuary (southwest Netherlands, 51°21′ N, 3°43′ E), where it represented the dominant grazer (~90% of all harpacticoid individuals). *M. littorale* specimens were extracted alive from the sediment using a mixed technique of sediment decantation and extraction via white light attraction. Adult specimens were randomly collected with a glass Pasteur pipette using a Wild M5 binocular (Joint Stock Company, Heerbrugg, Switzerland). Copepods were washed three times over a 38-μm sieve and placed in glass jars with filtered and autoclaved natural seawater (salinity 32 ± 1 psu) overnight in order to empty their intestines prior to the start of the experiment.

The diatom community was composed of six species representing the most abundant genera (i.e., *Nitzschia*, *Amphora*, *Cylindrotheca*, *Gyrosigma*, and *Navicula*) observed at the sampling site (Appendix S1: Table S1). All diatom species were obtained from the Diatom Collection of the Belgian Coordinated Collection of Micro-organisms culture hosted at the Protistology and Aquatic Ecology Research Group (Ghent University, Ghent, Belgium). Prior to the experiments, the diatoms were grown in tissue bottles (CELLSTAR TC, 175 cm^2 growth surface, CellStar, Greiner Bio-One, Frickenhausen, Germany) during 10 d in a climate room at 15° ± 1°C, a light/dark cycle of 12 h/12 h and an illumination of 90 μmol photons·m^{-2}·s^{-1}, in culture medium consisting of filtered and autoclaved natural seawater (salinity 32 ± 1 psu) enriched with f/2 nutrients (Guillard 1975). In spite of their potentially different optimal requirements, applying the same conditions (e.g., in terms of irradiance) was found to be appropriate (see previous experiments of De Troch et al.) as the species also co-occur in the field.

Diatom experiments

The experimental diatom communities were exposed to five treatments to 0, 200 (hereafter low), and 500 (hereafter high) μg/L atrazine and copper, respectively. Concentrations were based on trial tests (data not shown) as well as published sensitivity data for marine benthic diatoms (Pistocchi et al. 1997, Levy et al. 2007, Wood et al. 2014). Atrazine treatments were prepared from a stock solution obtained by dissolving 50 mg commercial atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 99.8% pure; Sigma-Aldrich Chemie Gmbh, Munich, Germany) in 10 mL acetone as a carrier to increase the solubility of atrazine, with a maximum final volume of 0.01% acetone in the treatments. An acetone control treatment of 0.01% acetone was included and compared to an acetone-free control to test for carrier effects. All atrazine treatments were compared to the acetone control. Copper (as a $Cu[II]Cl_2$ solution, analytical grade; VWR International, Leuven, Belgium) was spiked directly into the culture medium before exposure of the diatoms. F/2 culture medium was prepared without EDTA, to avoid complexation of free copper ions (Pistocchi et al. 1997). The obtained atrazine and copper concentrations that were finally applied in the experiment are listed in Appendix S1: Table S2. Additionally, the six diatom species were grown in monoculture under control conditions, to quantify each species' biomass and EFA production in the absence of the stressors (Appendix S1: Table S1). All treatments were run in tissue culture flasks (CELLSTAR TC, 175 cm^2 growth surface, CellStar, Greiner Bio-One), with nine replicates per treatment (three replicates in the monoculture treatments). Each microcosm (100 mL) was inoculated with a total cell density of approximately 5,000 diatom cells/mL (belonging to the same species in the monocultures, between 800 and 850 cells/mL per species in the diatom communities) from exponentially growing cultures, and incubated in a climate room at 15° ± 1°C, under a light:dark cycle of 12 h:12 h at 90 μmol photons·m^{-2}·s^{-1}. Culture medium was renewed after 8 and 15 d. Diatom biofilms as food for the copepod experiment as well as for the EFA analyses (see *EFA analyses*) were harvested after 15 d (late exponential growth phase). The experiments were terminated after 25 d.

Diatom biomass was quantified as biovolume after 0, 2, 5, 10, 15, 20, and 25 d of incubation. Biovolume was calculated from cell densities, linear dimensions (measured digitally using ImageJ; Schneider et al. 2012) and formulas representing the closest approximation of geometric shape for each genus (Hillebrand et al. 1999; Appendix S1: Table S1). Cell densities (in cells/mL) were determined by digitally counting the cells (ImageJ cell counting software) in photographs obtained by magnifying and photographing (×100) an area of 0.66 mm^2 per microcosm, using an inverted Axiovert 135 Zeiss microscope (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11, Canon, Diegem, Belgium). All analyses below use the biomass on day 15.

Diatom biofilms from three replicates per treatment were harvested as food for the corresponding treatments in the copepod experiment (see *copepod experiment*), and purified from copper and atrazine by centrifugation at 50 *g* for 10 min. The supernatant was replaced with f/2 culture medium, and the suspension was centrifuged again at 50 *g* for 10 min. A concentrated pellet containing 2.05 mm^3 diatom biovolume per replicate was transferred to nine Eppendorf microtubes (0.23 mm^3 per microtube), freeze-dried, and preserved at −80°C. The individual microtubes contained the food aliquot for each day of the respective treatments in the copepod experiment (see *copepod experiment*).

Copepod experiment

We tested the effect of diatom diet quality by offering *M. littorale* diatom diets of equal biomass under unstressed conditions. The copepod experiment consisted of five treatments, each with three replicates of 100 *M. littorale* copepods (a natural mix of adult males and [gravid] females), that were fed for 10 d an equal biomass of diatoms grown under unstressed conditions and low and high atrazine and copper stress, respectively. The experiment was conducted in glass jars containing 100 mL of filtered and autoclaved seawater in a climate room at 15° ± 1°C with a 12:12 h light:dark cycle and 40–50 μmol photons·m^{-2}·s^{-1}. To ensure a constant food supply, each treatment was inoculated with a concentrated diatom pellet of 0.23 mm^3 biovolume every day, and unconsumed diatoms were removed from the bottom of the jars. Over the duration of the experiment, a total diatom biovolume of 2.05 mm^3 was applied per experimental unit, corresponding to 3–5 × 10^6 diatom cells per treatment. At the end of each day, there was no food depletion in any of the treatments. Based on our previous experiments (De Troch et al. 2005, 2007), the provided quantity of diatoms can be considered as above the feeding saturation level. Copepod mortality was determined at the end of the experiment, and surviving (85–100%) individuals from each experimental unit were washed in natural seawater to remove food particles, left 12 h to empty their gut, and stored at −80°C for further fatty acid analysis.

EFA analyses

Essential fatty acids (EFAs) as a marker of diatom diet quality were quantified as the content of eicosapentaenoic acid (20:5ω3, EPA) and docosahexaenoic acid (22:6ω3, DHA). These EFAs were selected as proxies to quantify the energy transfer in view of their relevance for the next trophic level (copepods). Three replicates of 8 mL suspended diatom culture per treatment of the diatom communities and monocultures were collected after 15 d. The samples were centrifuged for 10 min at 10°C at 50 *g*. After undergoing the same purification process as the diatom food samples for the grazer experiment, pellets were resuspended, placed in a glass vial and stored at −80°C for fatty acid analysis.

Copepod and diatom EFA content was measured through hydrolysis of total lipid extracts and methylation to FA methyl esters (FAME), followed by the analysis of the obtained FAME using a gas chromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973) according to the protocol described in De Troch et al. (2012a) for copepods and Mensens et al. (2015) for diatoms. The quantification function of each individual FAME was obtained by linear regression of the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 ng/mL). All EFA concentrations were standardized to diatom

biomass (see *diatom experiments*) or the number of copepod individuals for diatoms and copepods, respectively.

Data analysis

An analysis of the diatom community structure among the treatments (control, low and high atrazine and copper) was conducted with a nonmetric multidimensional scaling method based on Bray-Curtis similarity. A one-way analysis of similarity (ANOSIM) was used to test for significant biomass differences between the treatments. Subsequently, percentages of similarity (SIMPER) were calculated to determine the main species contributing to any differences in community structure.

Differences in diatom biomass and EFA production among the treatments were tested with a generalized least squares model, with biomass and EFA production as response variables and treatment type as categorical predictor

$$Y \sim \beta_T \times T \tag{1}$$

where Y is the response variable (biomass or EFA production per unit biomass), T is the treatment type (control, low and high atrazine and copper), and the beta coefficient β_T is the slope measuring the effect of the treatment type on biomass or EFA production. Biomass and EFA production among treatments were compared with pairwise Tukey's tests correcting P values for multiple comparisons by the single-step method.

Next, we tested if potential changes in diatom EFA production were due to selective or context-dependent effects of atrazine and copper. Selective and context-dependent effects were quantified by comparing the EFA production in experimental and synthetic diatom communities. The synthetic communities have the same community structure as observed in the corresponding treatments of the experimental communities, but are computed from the EFA production in unstressed monocultures of each species (Eq. 2). The synthetic communities thus reflect the EFA production expected at the same community structure as induced by copper and atrazine, however without any stress exposure or species interactions.

$$Y_{\mathrm{SYN},j} = \frac{\sum_{i=1}^{N} M_{i,j=0} \times B_{i,j}}{B_{T,j}}. \tag{2}$$

$Y_{\mathrm{SYN},j}$ is the EFA production per unit biomass in synthetic communities of the same structure as the experimental communities at atrazine or copper concentration j. $M_{i,j=0}$ is the mean EFA production per unit biomass of species i in monoculture under unstressed conditions ($j = 0$). $B_{i,j}$ is the biomass of species i observed in the experimental community at the stress level j. $B_{T,j}$ is the total biomass observed in the experimental community at stress level j.

Selective and context-dependent effects of both stressors were analysed with a generalized least squares model (Eq. 3) and pairwise comparisons of the EFA production in experimental and synthetic diatom communities

$$Y \sim \beta_T \times T \tag{3}$$

where Y is the diatom EFA production per unit biomass, T is the treatment type (control, low and high atrazine and copper in the experimental and synthetic diatom communities), and the beta coefficient β_T is the slope measuring the effect of the treatment type on EFA production. Pairwise comparisons were performed with a Tukey's test correcting P values for multiple comparisons by the single-step method.

Context-dependent effects occur when synthetic and experimental communities within the same treatment differ in their EFA production per unit biomass. Since both community types have the same structure, any differences in EFA between the two community types result from direct stress effects or species interactions in the experimental community. Consequently, any differences between experimental and synthetic communities of the same treatment point to context-dependent effects.

Selective stress effects occur when synthetic communities reflecting the control community structure differ in their EFA production from synthetic communities reflecting the community structure under stress. Since EFA values for synthetic communities are computed from those of unstressed monocultures, any differences between synthetic communities are related to differences in community structure rather than direct stress effects or species interactions. Consequently, any differences among synthetic communities are linked to selective rather than context-dependent effects. Appendix S1: Fig. S1 provides a scheme visualizing the quantification of context-dependent and selective stress effects.

The response of copepod fatty acid content to stressor-induced alterations in the quality and community structure of their diatom diet was analysed with generalized least squares models, with copepod fatty acid content as response variable and diatom diet quality and community structure as predictors (Eq. 4). Diatom diet quality was quantified as EFA production, diatom community structure as the Bray-Curtis percent similarity to the average community structure in the controls (Eq. 5). Models were fitted separately for copepods feeding on atrazine- and copper-exposed diatoms respectively, to test if the effects of either stressor on copepod fatty acid content can be predicted from changes in diatom diet quality or community structure

$$E_{\mathrm{C}} \sim a + b \times E_{\mathrm{D}} + c \times C_{\mathrm{D}} \tag{4}$$

where E_{C} is the copepod fatty acid content (EFA content per copepod individual), E_{D} is diatom diet quality (EFA production per unit biomass), C_{D} is diatom community structure (see Eq. 5), a is the intercept, and b and c represent the slopes, i.e., the relation of copepod EFA content to diatom diet quality and diatom community structure. If E_{D} and C_{D} were correlated (correlation factor >0.5), models were fitted separately for both predictors

$$C_{\mathrm{D}} = 100 \times \frac{1 - \sum |B_{i,j} - \mu B_{i,j=0}|}{\sum (B_{i,j} + \mu B_{i,j=0})} \tag{5}$$

where $B_{i,j}$ is the biomass of species i at the atrazine or copper concentration j and $\mu B_{i,j=0}$ is the mean biomass of species i in the control ($j = 0$).

For all least squares model fits, normality and homogeneity of model residuals were inspected by evaluation of quantile-quantile plots and Shapiro-Wilk's test and by Levene's test and plotting residuals vs. explanatory variables, respectively. Untransformed data did not violate normality (Shapiro-Wilk's test, $\alpha > 0.1$). If indications of deviations from normality were detected ($0.1 < \alpha < 0.15$), an optimal Box-Cox transformation was applied to maximize normality of model residuals (Box and Cox 1964, Venables and Ripley 2002). If homogeneity was violated, the model was refitted using an exponential variance structure allowing residuals to change with the continuous predictor X (weights = varExp (form $\sim 1|X$) or allowing different variances according to the categorical predictor P (weights = varIdent(form $\sim 1|$P). By means of likelihood ratio testing, the significance of these structures was tested ($\alpha = 0.05$).

Multivariate, ANOSIM, and SIMPER analyses of diatom community structure were performed using Primer 6 software (Clarke and Gorley 2006). All other calculations were done in R 3.0.1. using RStudio (R Development Core Team 2016). The package nlme (Pinheiro et al. 2016) was used for the fitting of generalized least squares models and optional variance structures. Optimal Box-Cox transformations were performed using MASS (Venables and Ripley 2002). Pairwise Tukey's tests on the fitted models were performed with the package multcomp (Hothorn et al. 2008), using the general linear hypothesis test (glht) function, correcting P values for multiple comparisons by the single-step method (default procedure in multcomp).

Results

Diatom community structure

The structure of diatom communities under atrazine differed from the structure of communities grown under control conditions and copper exposure (see nonmetric multidimensional scaling in Appendix S1: Fig. S2, ANOSIM global $R = 0.833$, $P = 0.001$). The community structure at both copper levels resembled the community structure observed under control conditions (14% and 18% dissimilarity, respectively), with *N. acicularis* and *N. arenaria* contributing most to biomass in both types of communities (Fig. 1; Appendix S1: Table S3). In contrast, both atrazine levels induced a change in community structure (70% and 76% dissimilarity from the control, Appendix S1: Table S3) due to an increase in biomass of *C. closterium*, which compared to the control showed a 6- and 12-fold increase in biomass in the high and low atrazine treatments, respectively. This resulted in a dominance by *C. closterium* in the atrazine-exposed communities, as it contributed more than 70% of the total biomass at both atrazine levels (Fig. 1; Appendix S1: Table S4). Within the control, copper, and atrazine treatments, the community structure of diatom communities showed little variance (within-treatment similarities between 84% and 92%; Appendix S1: Table S4).

Diatom biomass and EFA production

Diatom biomass and EFA production changed depending on treatment type (all $P < 0.0001$, Figs. 1, 2). The post-hoc analyses showed that diatom biomass was reduced at both low and high copper as well as at high but not at low atrazine concentrations (Fig. 1, Table 1). Diatom EFA production was reduced at both levels of atrazine, but only at high copper stress (Fig. 2, gray bars, Table 1). High copper stress reduced the EFA production of diatom communities by 40%, low and high atrazine stress by 60% and 75%, respectively (gray bars in Fig. 2).

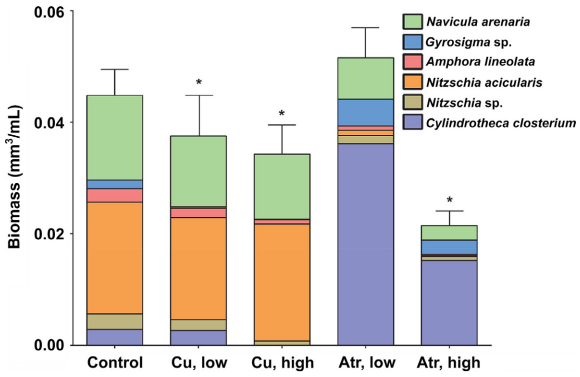

FIG. 1. Total biomass production per treatment and biomass of the component species for diatom communities grown in control, low (200 μg/L) and high (500 μg/L) atrazine (Atr) and copper (Cu) treatments. Significant differences ($P \leq 0.05$) in biomass production from the control are indicated with asterisks (*). Bars show mean biomass, error bars are the standard deviation.

Selective and context-dependent stress effects on diatom EFA production

The EFA production in the experimental and synthetic diatom communities changed depending on treatment type

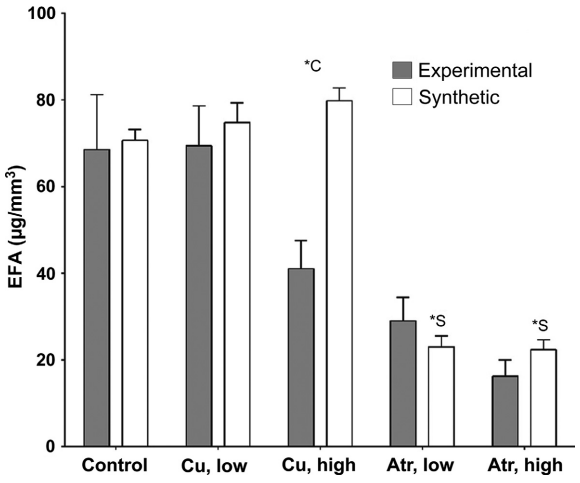

FIG. 2. Atrazine and copper effects on diatom diet quality (EFA production). Experimental communities were grown in control, low (200 μg/L) and high (500 μg/L) atrazine (Atr) and copper (Cu) treatments. Synthetic communities were computed from unstressed monocultures in the same community structure observed at each stress level. Significant differences ($P \leq 0.05$) in EFA production indicating selective and context-dependent stress effects are indicated with asterisks (*). *C indicates pairwise differences among stressed and synthetic communities of the same structure (context-dependent stress effects). *S indicates pairwise differences among control and stress treatments within the synthetic communities (selective stress effects). Values are mean EFA concentrations and bars are the standard deviation.

TABLE 1. Pairwise comparisons of biomass and EFA production in diatom communities as estimated by generalized least squares model fits.

Treatment	Treatment	Est	lwr	upr	*z*	*P*
Biomass						
Control	Atr, low	−6.756	−9.18	−4.33	2.78	0.059
Control	Atr, high	23.327	20.90	25.75	9.61	**<0.001**
Control	Cu, low	7.273	4.85	9.70	−3.00	**0.036**
Control	Cu, high	10.533	8.11	12.96	4.34	**<0.001**
Atr, low	Atr, high	30.083	27.66	32.51	12.39	**<0.001**
Cu, low	Cu, high	3.260	0.83	5.69	1.34	0.666
EFA production						
Control	Atr, low	2.143	1.30	2.99	8.00	**<0.001**
Control	Atr, high	3.191	2.34	4.04	12.08	**<0.001**
Control	Cu, low	−0.046	−0.89	0.80	−0.17	1.000
Control	Cu, high	1.372	0.52	2.22	5.12	**<0.001**
Atr, low	Atr, high	0.317	0.13	0.51	5.72	**<0.001**
Cu, low	Cu, high	0.305	0.11	0.50	4.91	**<0.001**

Notes: Biomass or EFA production indicate to which response variable models were fitted. "Treatment" (low [200 μg/L] and high [500 μg/L] atrazine [Atr] and copper [Cu]) indicates which treatments are compared. "Est" indicates the difference in biomass (in 10^3 mm^3/mL), and EFA (in μg/mm^3) production between the compared treatments as estimated by generalized least squares models fitted to untransformed biomass and Box-Cox transformed EFA data, "lwr" and "upr" indicate the lower and upper confidence intervals of the estimated difference. The *z* and *P* values corrected for multiple comparisons by the single-step method are presented; values in boldface type are statistically significant ($P \leq 0.05$).

(both $P < 0.0001$). Pairwise comparisons of the EFA production in experimental and synthetic communities did not show differences among the two community types in the control and low copper treatments (Fig. 2, Table 2). At high copper stress, the EFA production in the experimental community was lower than in the corresponding synthetic community (Fig. 2, Table 2). The EFA loss induced by high copper stress in experimental communities was thus not reflected in unstressed synthetic communities of the same structure, whose EFA production did not differ from the control (Fig. 2, Table 2).

In the atrazine treatments, both the experimental and synthetic communities had a lower EFA production than the control (Fig. 2, Table 2). Within each atrazine treatment, the EFA production of experimental and synthetic communities did not differ (Fig. 2, Table 2). The EFA loss induced by atrazine in the experimental communities was thus reflected by the synthetic communities, which mimicked the community structure under atrazine without actual exposure to the herbicide (Fig. 2, Table 2).

Diet quality effect on copepods

The EFA content of *M. littorale* was related to the stressor-induced changes in diatom diet quality (both stressors) and diatom community structure (atrazine only; Fig. 3, Table 3). Copepods maintained their control EFA content when feeding on diatoms from the low copper treatment, but lost half of their EFAs when feeding on diatoms grown under high copper stress (Fig. 3). This resulted in a positive correlation of copepod EFA content and diatom EFA content (Table 3). When offered diatoms from the low and high atrazine treatments,

TABLE 2. Pairwise comparisons of EFA production in treatments of experimental and synthetic diatom communities as estimated by generalized least squares model fits.

Effect	Com	Treatment	Com	Treatment	Est	lwr	upr	*Z*	*P*
SE	syn	control	syn	Atr, low	2.695	1.85	3.54	10.06	**<0.001**
SE	syn	control	syn	Atr, high	2.746	1.90	3.60	10.25	**<0.001**
SE	syn	control	syn	Cu, low	−0.173	−1.02	0.67	−0.65	0.999
SE	syn	control	syn	Cu, high	−0.381	−1.23	0.47	−1.42	0.921
CD	exp	control	syn	control	−0.110	−0.96	0.74	0.41	1.000
CD	exp	Atr, low	syn	Atr, low	0.442	−0.41	1.29	1.65	0.823
CD	exp	Atr, high	syn	Atr, high	−0.554	−1.40	0.29	−2.07	0.550
CD	exp	Cu, low	syn	Cu, low	−0.237	−1.08	0.61	0.89	0.997
CD	exp	Cu, high	syn	Cu, high	−1.863	−2.71	−1.02	−6.95	**<0.001**

Notes: Types of stress analyzed are selective stress effects (SE; comparison of synthetic communities reflecting the community structure under control and stress conditions) and context-dependent stress effects (CD; comparison of stressed and synthetic communities of the same community structure). Communities (experimental [Exp] and synthetic [Syn]) and treatments (low [200 μg/L] and high [500 μg/L] atrazine [Atr] and copper [Cu]) indicate which communities and treatments are compared. Est indicates the difference in EFA production between the compared treatments as estimated by generalized least squares models fitted to Box-Cox transformed EFA data; lwr and upr indicate the lower and upper confidence intervals of the estimated difference. The *z* and *P* values corrected for multiple comparisons by the single-step method are presented; values in boldface type are statistically significant.

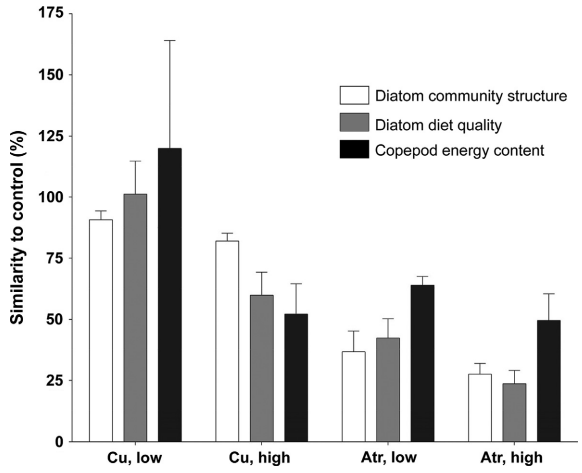

FIG. 3. Community structure and diet quality (EFA production per unit biomass) of diatom communities exposed to stress and EFA content of *Microarthridion littorale* after 10 d of feeding on the respective diets, visualized as percent similarity to the corresponding control. The similarity of diatom community structure is calculated as the Bray–Curtis similarity to the control mean (see Methods). The similarity of diatom and copepod EFA content is calculated as fraction percentage of the EFA concentration in treatment i and the mean (μ) EFA concentration in the corresponding control c: $(EFA_i/\mu EFA_C) \times 100$. Diatom communities were exposed to low (200 μg/L) and high (500 μg/L) atrazine (Atr) and copper (Cu) concentrations, copepods were not exposed to any of the stressors. Values are means and bars are the standard deviation.

copepods also lost half of their EFA content, which was predicted not only by the diatoms' EFA content, but also by the changes in diatom community structure (Fig. 3, Table 3). Appendix S1: Fig. S3 shows the EFA content per diatom biomass and per copepod individual, as well as the relative proportion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In diatoms, the essential fatty acids consisted mainly of EPA, whereas DHA was the main component in copepods (EPA : DHA ratio 4–6 in diatoms, 0.3–0.6 in copepods; Appendix S1: Fig. S3). The copepod survival rate in the experimental units was between 85% and 100%. The EFA content of copepods feeding on diatoms from control conditions (65.3 ± 6.1 ng/copepod [mean ± standard deviation]) did not differ significantly from copepods at the start of the experiment (i.e., animals collected in the field, 68.8 ± 11.8 ng/copepod, t test, $P = 0.99$, Appendix S1: Fig. S3).

Discussion

Biomass production is the most widespread functional endpoint in research on ecosystem functioning under anthropogenic change, and producer biomass (diet quantity) has been the main focus in most trophic experiments (Arts and Wainmann 1999, Balvanera et al. 2006, Cardinale et al. 2011). The loss of diatom biomass induced by copper corresponds to previous findings on the toxicity of copper to marine diatoms (Pistocchi et al. 1997, Masmoudi et al. 2013). Diatom biomass was not affected at low atrazine concentrations, although atrazine has been shown to reduce diatom biomass at concentrations lower than those used in our study (DeLorenzo et al. 1999, 2001, Magnusson et al. 2008). The capacity of our experimental diatom communities to maintain their biomass under low atrazine exposure was related to a change in community structure, with *C. closterium* becoming dominant in all atrazine-stressed communities. Atrazine blocks the electron transport chain of Photosystem II (PSII; Dorigo and Leboulanger 2001). Some diatom species can however reduce their dependency on photosynthesis and thus their sensitivity to PSII inhibitors by mixotrophic growth (i.e., the uptake of organic substrates; Debenest et al. 2009, Larras et al. 2014). *C. closterium* is capable of mixotrophic growth (Vanelslander et al. 2009), which reduces its sensitivity to herbicide stress (Mensens et al. 2017). The

TABLE 3. Results of generalized least squared models predicting copepod EFA content from diet quality (diatom EFA production) and diatom community structure.

Stressor, predictor, and model	Slope	SE	T	P	AIC	Log likelihood	Validity	LR	P LR
Atrazine									
EFA								0.69	0.41
1	0.059	0.008	7.38	**0.0002**	28.46	−11.23	yes		
2	0.060	0.007	9.06	**<0.0001**	29.77	−10.89	yes		
Comp								0.07	0.79
1	0.045	0.008	5.88	**0.0006**	31.69	−12.85	yes		
2	0.045	0.008	5.64	**0.0008**	33.62	−12.81	yes		
Copper									
EFA								3.64	0.06
1	0.137	0.021	6.57	**0.0003**	35.21	−14.61	yes		
2	0.133	0.014	9.17	**<0.0001**	33.57	−12.79	yes		
Comp								0.75	0.39
1	0.204	0.126	1.62	0.1486	44.82	−19.41	no		
2	0.071	0.150	0.48	0.6494	46.07	−19.03	yes		

Notes: Copepods were offered a diet of diatom communities exposed to copper or to atrazine. Predictors are diet quality in terms of diatom community composition (Comp) or EFA production. Model indicates if the model was fitted without (Model 1) or with (Model 2) variance structure. Slope indicates the relation between predictors and copepod EFA content, i.e., the effect of diatom diet quality and community structure on copepod EFA content. SE is the standard error on the estimated slopes. The t and P values are presented; values in boldface type are statistically significant. AIC is the Akaike information criterion. Validity denotes if residuals were homogeneous and normally distributed or not (yes or no). If no, models were refitted (Model 2) with a variance structure allowing the residuals to change with the predictor. LR is the likelihood ratio of model 1 vs. model 2; P LR the corresponding P value.

presence of a mixotrophic, atrazine-tolerant species thus caused changes in community structure that underpinned the absence of biomass loss at low atrazine levels.

Selective atrazine stress determined the diet quality of diatom communities, which lost 60% and 75% of their EFA content at low and high levels of the pesticide. The same extent of energy loss was observed in synthetic communities that reflected the community structure under atrazine without exposure to the pesticide. As diet quality in atrazine-exposed experimental communities and unexposed synthetic communities did not differ, the energy loss under atrazine was caused by selective changes in community structure rather than by context-dependent atrazine effects on diatom diet quality. This selective atrazine stress could be attributed to the dominant species *C. closterium*, which produced three and eight times less EFAs than the species contributing the most biomass under control conditions, i.e., *N. arenaria* and *N. acicularis*, respectively (see fatty acids per species in Appendix S1: Table S1). In the presence of copper, communities were dominated by the same lipid-rich species as in the control, and as a result no selective effects on diet quality were observed. The lower diet quality in experimental compared to synthetic communities at high copper levels was therefore caused by context-dependent rather than selective copper effects. Context-dependent effects also likely caused the further loss of EFAs at high compared to low atrazine stress, since community structure at the two herbicide levels did not differ. This loss of diet quality at high levels of both stressors could be due to physiological stress effects such as an alteration of photosynthesis and thus of the carbon supply for fatty acid synthesis, inhibition of the enzymes involved in lipid biosynthesis or an increase in the degree of fatty acid saturation, which are all reported to reduce the microalgal EFA production under metal and pesticide stress (Böger et al. 2000, Guschina and Harwood 2006, Chia et al. 2013).

In our study system, selective stress proved to be the main driver of microalgal diet quality. Selective atrazine stress caused a more important loss of diatom diet quality than the context-dependent effects of both stressors, at chemical concentrations where no context-dependent effects on individual EFA content were recorded. It should however be highlighted that the levels of metal and pesticide stress used in this work are unlikely to occur in the diatoms' natural habitat. The atrazine and copper concentrations in this study can be representative of polluted estuaries in Asia and North America (Bai et al. 2011, Smith et al. 2012), but do not represent reported field concentrations in Europe (Janssen et al. 2010, Nödler et al. 2013), making chemical-stress-induced losses of diatom or copepod species in North Sea intertidal systems highly unlikely (Masmoudi et al. 2013, Wood et al. 2014). However, in this work chemical stress reduced ecosystem functioning mainly indirectly through shifts in community structure toward dominance by unproductive species rather than by directly affecting the species' numbers or their contribution to functioning. Such shifts in community structure can be induced by chemical concentrations lower than those used in this work (Debenest et al. 2010) and anthropogenic stressors are causing large-scale shifts in the composition of aquatic communities, with concomitant impacts on the functioning of aquatic food webs (Hicks et al. 2011, McMahon et al. 2012, Litchman et al. 2015). Due to the pronounced differences in lipid profiles within and among algal classes (Taipale et al. 2013, Guo et al. 2016), these changes in community structure rather than direct stress effects on algal biochemical profiles could represent a potentially stronger driver of trophic energy flow under anthropogenic change.

The EFA content of the copepod *M. littorale* closely tracked that of its diet. Selective and context-dependent stress effects on diatom diet quality resulted in a concomitant loss in the EFA content of their main copepod grazer, confirming algal EFAs as being a key component of diet quality that is directly linked to trophic energy transfer. The DHA : EPA ratio of *M. littorale* was higher than in the diatom communities, which corresponds to previous findings on the relative concentrations of both EFAs in copepods and their algal diets (De Troch et al. 2012a, Arndt and Sommer 2014). Diatoms are characterized by a high EPA content (Taipale et al. 2013, Guo et al. 2016), but DHA appears to be the most important fatty acid for copepods (Taipale et al. 2013). Planktonic primary consumers such as cladocerans or calanoid copepods directly depend on the DHA taken up from their diet (Bell et al. 2007, Bell and Tocher 2009, De Troch et al. 2012a), but several harpacticoid copepod species are able to bioconvert EPA to the longer chain DHA, a capacity that has notably been demonstrated in *M. littorale* (De Troch et al. 2012a). While the total EFA content of *M. littorale* reflected that of its different diatom diets, this capacity to convert EPA to DHA likely enabled *M. littorale* to maintain high relative levels of DHA.

Our design of calculating synthetic communities from unstressed monocultures eliminated diversity effects such as species interactions, which can drive the functional contribution of communities along environmental gradients (Tylianakis et al. 2008a, Maestre et al. 2010). It should thus be noted that, while our experiments highlight potential functional impacts of selective stress, they do not allow to quantify diversity effects on diatom functioning. Our results also have to be treated carefully due to the limited number of stress levels and replicates. Also, offering *M. littorale* preserved rather than live diatom food might have influenced food uptake. Freeze-drying does not alter the biochemical composition within diatoms cells, but modifies the exterior of diatom cells through the loss of exudates or bacteria associated to the diatom frustule, which can affect the ingestion of diatoms by harpacticoid copepods (Cnudde et al. 2011). Feeding *M. littorale* live diatom cultures under unstressed conditions would however have resulted in a dissimilar diatom community structure than that induced by the stressors: atrazine and copper did not eliminate any of the diatom species, but caused alterations of community evenness, which typically cannot be maintained in the absence of the stressors (De Laender et al. 2016).

Since their EFA content ranks among the highest of all algae classes, diatoms are regarded as a high-quality food source and a crucial link for the energy flow at the basis of aquatic food webs (Guo et al. 2016). Here, diatom diet quality was more affected by chemical stress than by diet quantity. The selective and context-dependent stress effects on diatom diet quality were caused by the large interspecific differences in EFA content and the loss of diatom EFA

content under stress. Indeed, the diet quality of benthic diatoms shows more interspecific variation and is more affected by chemical pollutants than their contribution to diet quantity (Mensens et al. 2015). Losses in diet quality occurred at copper concentrations that also impacted the survival of *M. littorale* when the copepods where directly exposed to copper (Appendix S1: Table S6). In addition to indirect effects on copepod EFA content through changes in diet quality, high metal stress can thus be expected to reduce copepod abundance through direct toxic effects. Conversely, atrazine changes microalgal community structure at concentrations lower than the 200 μg/L used in our study (Bérard and Benninghoff 2001, Debenest et al. 2010), whereas atrazine has been found to only acutely affect copepods at concentrations higher than 1 mg/L (Hall et al. 1995, Bejarano and Chandler 2003). Atrazine could thus first affect copepods indirectly through selective changes in the structure of their diatom diet rather than through direct effects on the copepods themselves.

The loss of diet quality did not result in increased harpacticoid mortality. Low diet quality rarely causes acute copepod mortality, but reduces copepod EFA content and, in the longer term, growth rate and reproduction (Müller-Navarra 1995, Müller-Navarra et al. 2000, Arendt et al. 2005, Gonçalves et al. 2011). The EFA content of copepods is crucial for their main consumers, especially larval fish whose development can depend on the EFAs taken up from their copepod prey (Sargent et al. 1995, Payne et al. 1998). While, in this study, losses in diet quality did not eliminate consumers, low diet quality could thus reduce the energy transfer at the plant-animal interface, which is a key limiting factor for the functioning of aquatic ecosystems (Brett and Müller-Navarra 1997, De Troch et al. 2012b). Nonetheless, the importance of algal diet quality, as distinct from just diet quantity, is rarely highlighted in research on food web functioning (Guo et al. 2016). Due to its sensitive response to selective stress, algal diet quality in terms of EFA production and community structure provides a powerful approach to integrate our understanding of coastal ecosystem functioning under anthropogenic change.

Our results support two conclusions. First, chemical stress differentially affects the contribution of marine primary producers to diet quantity and diet quality, with diet quality being more sensitive in this study. Second, selective stress caused a more important loss of diatom diet quality in our study system than context-dependent stress effects. Rather than variations in physiology, changes in community structure and differences in the diatoms' biochemical profiles thus represented the main driver of for energy flow to their copepod consumers. Moreover, it can be expected that the type of stress tested in this experiment will not only affect the copepod energy content, but also their growth and reproductive physiology. Therefore, the integration of diet quality into traditional studies of diet quantity is recommended to assess energy flow in marine food webs under anthropogenic change.

Acknowledgments

The first author acknowledges the Doctoral Programme on Marine Ecosystem Health and Conservation (MARES) and the UGent Special Research Fund (BOF) for his doctoral research fellow grant. The authors received additional financial support from the research council of Ghent University in the form of project BOF-GOA 01GO2617. The fatty acid analyses were performed by Dirk van Gansbeke and supported by FWO-Flanders in the form of the research grant 1523814N awarded to the last author.

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